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International application number: PCT/SE04/001872

International filing date: 15 December 2004 (15.12.2004)

Document type: Certified copy of priority document

Document details: Country/Office: SE
Number: 0303581-3
Filing date: 23 December 2003 (23.12.2003)

Date of receipt at the International Bureau: 07 January 2005 (07.01.2005)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)

PRV

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Patentavdelningen

PCT / SE 2004 / 0 0 1 8 7 2

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(21) *Patentansökningsnummer* / *Patent application number* — *0303581-3*

(86) *Ingivningsdatum* / *Date of filing* — *2003-12-23*

Stockholm, 2004-12-20

För Patent- och registreringsverket
For the Patent- and Registration Office

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NOVEL USE OF POSITIVELY CHARGED SUPPORT

Technical field

The present invention relates to a novel use of a positively charged support. More closely, the invention relates to a sample loading support paper or membrane for loading samples onto an electrophoretic gel. The support is provided with positively charged groups and is used to load samples from the cathode side of the electrophoretic gel.

Background

One type of widely used electrophoresis is isoelectric focussing, wherein substances, such as proteins, are separated according to their pI-value. For isoelectric focussing, sample loading has traditionally been performed by cup loading by placing a cup on the gel and letting a sample pass through the cup into the gel. The cup is positioned on the gel for the whole electrophoresis run.

Alternatively for dried gels, the sample may be mixed with electrophoresis buffer and used as a rehydration solution to rehydrate the dried gel, such as Immobiline DryStrip™ gels.

More recently, sample application paper in the form of conventional filter paper, has been placed between the electrode and the electrophoresis gel to load a sample into an electrophoretic gel. This functions satisfactorily for sample application from the anode side of the gel. However, this approach does not work when using acidic pH intervals. As an alternative, rehydration loading can be used in these pH intervals.

However, rehydration loading is not possible with swollen gels, such as pre-swollen RTG (ready-to-go) strips, Thus, these kind of gels need an alternative loading, especially for application of large samples which is very difficult today.

Supports provided with positively charged groups are known within prior art.
For example, US 3 714 010 describes anion exchange membranes from cellulosic sheet materials such as cellophane, parchment paper or kraft paper. The membrane is especially suited for use in the electrodialytic purification of saline water.

US 4 080 171 describes a method for analysis of trace components in a liquid, which comprises filtering said liquid through a filter paper having at least one anion exchange.

US 5 151 189 describes a cationic charge modified microporous membrane. This membrane can be used in various applications such as filtration of fluids and macromolecular transfer from electrophoresis gels. The transfer process, also known as "blotting", is defined herein as the steps involved in physically moving biomolecules from a gel matrix to a microporous membrane onto which they become immobilised.

The most common prior use of anion exchange supports within prior art has been the use of cellulose anion exchange paper for chromatography purposes. Examples of this are DEAE- cellulose paper and aminoethyl-cellulose paper.

According to our knowledge there is no prior art describing electrophoresis sample loading with positively charged support.

Summary of the invention

A first objective of the invention was to provide an alternative way to load samples onto electrophoretic gels.

A second object of the invention was to enable sample loading from the cathode side of an electrophoretic gel, such as an IPG (Immobilised pH gradient) gel or strip.

A third objective of the invention was to enable sample application to an acidic interval IPG gel or strip, such as a RTG (ready-to-go) strip.

A fourth object was to enable sample loading in preparative amounts of protein.

These and other objectives were achieved according to the invention by providing use of a positively charged support for sample application from the cathode side of the gel. Thus, the invention provides a new method of using a positively charged support.

Thus, in a first aspect, the invention relates to use of a hydrophilic support derivatised with positively charged groups, for sample application to electrophoretic gels, such as IPG (Immobilised pH gradient) gels. According to the invention the application is performed from the cathode side of the electrophoretic gel.

The support is preferably made of regenerated cellulose, dextran, agarose, polyvinylalcohol, polyether sulfone, polysulfone, cellulose acetate, polyurethane, polyamide, nylon or other types of membranes and composite membranes.

Preferably, the positively charged groups are cation groups. The degree of substitution with cation groups on the support may not cause adsorption of substances present in the sample, such as proteins, to the support.

Preferably, the cation groups are quartenary groups, such as QAE or Q groups, or DEAE.

A preferred support is made of regenerated cellulose substituted with a low degree of quaternary groups, preferably Q-groups.

In a preferred embodiment, the IPG gel is an acidic interval (such as pH 3.5-5) IPG gel or strip. One type of preferred IPG strips are RTG (ready-to-go) strips. RTG-strips are pre-swollen gels available in different pH-intervals.

The sample applicator according to the invention may be used in analytical as well as preparative amounts, a preferred use is for application of samples in preparative amounts.

The sample applicator may be used for application of samples to IPG gels per se or used for 2D gels, wherein the first dimension is isoelectric focussing and the second dimension is according to molecular weight.

In a second aspect, the invention relates to a kit comprising a positively charged sample application support according the above and an IPG gel, preferably a pre-swollen RTG strip, and more preferably an acidic interval RTG-strip, such as pH 3.5-5, pH 3.5-4.5 or pH 4-5.

In a third aspect, the invention relates to a sample applicator for electrophoresis comprising regenerated cellulose derivatised with Q-groups.

Detailed description of the invention

The present invention provides novel use of a positively charged support, namely as a sample applicator in electrophoresis. According to the invention the support is a hydrophilic support with high water absorbing capacity. Preferably the support can hold a large sample volume, such

as 1 ml sample. The amount of sample added to the support is usually from 50 µl – 10000 µl in a concentration of up to 10 mg/ml. The support must be substantially inert to the substances, such as proteins, present in the sample.

The support is made of any material with high water absorbing capacity, such as, but not limited to, regenerated cellulose, dextran, agarose, polyvinylalcohol, polyether sulfone, polysulfone, cellulose acetate, polyurethane, polyamide, nylon or other types of membranes and composite membranes.

According to the present invention, the support is substituted with positively charged cation groups, such as DEAE (diethylaminoethyl) or quaternary groups (for example Q (quaternary ammonium) or QAE (quaternary aminoethyl) groups) to give the paper a positive charge and anionic exchange character. This support can be used for application of samples from the cathode side of the gel.

The technique for derivatising the support or paper is known per se and can be found, for example, in "Membrane chromatography: Preparation and Applications to Protein Separation" Xianfang Zeng, Eli Ruckenstein; Biotechnol. Prog. 1999, 15, 1003-1019.

A preferred support is made of regenerated cellulose (paper) substituted with a low degree of quaternary ammonium groups, preferably Q-groups.

The thickness of the support depends on the support material. For regenerated cellulose (paper) the thickness is preferably 3-4 mm. The dimensions of the support are determined by the size of the gel and the sample amount.

The sample loading support according to the invention may be used in association with any swollen electrophoretic gel, preferably an IPG gel.
The sample is added to the support and thereafter it is placed between the cathode and the electrophoresis gel. At one end the support is in contact with the cathode and at the other end in contact with the cathode side of the gel. The running conditions are the same as for any IPG run or 2D electrophoresis run.

When using conventional cup loading, there are often disturbances in the first 15% of the gradient due to the presence of the cup. For short IPG strips this may be a very significant portion of the gel. With the present invention this problem is avoided.

The sample may be loaded in analytical or preparative amounts. The sample may be a biological sample or any other sample.

The present invention is especially suited for application of large sample amounts up to 1 ml and up to 10 mg/ml and is therefore very useful for preparative runs of large amounts of sample, preferably large amounts of protein.

CLAIMS

1. Use of a hydrophilic support derivatised with positively charged groups, for sample application to electrophoretic gels.

2. Use according to claim 1, wherein the sample application is performed from the cathode side of the electrophoretic gel.

3. Use according to claim 1 or 2, wherein the electrophoretic gel is an IPG (Immobilised pH gradient) gel.

4. Use according to claim 1, 2 or 3, wherein the support is made of regenerated cellulose, dextran, agarose, polyvinylalcohol, polyether sulfone, polysulfone, cellulose acetate, polyurethane, polyamide, nylon or other types of membranes and composite membranes.

5. Use according to one or more of the above claims, wherein the positively charged groups are cation groups.

6. Use according to claim 5, wherein the cation groups are quaternary groups.

7. Use according to claim 6, wherein the quaternary groups are QAE or Q groups.

8. Use according to claim 5, wherein the cation groups are DEAE- groups.

9. Use according to one or more of the above claims, wherein the electrophoretic gel is an acidic interval IPG gel.

10. Use according to any of the above claims, wherein the IPG gel is a pre-swollen RTG (ready-to-go) gel.

11. Use according to one or more of the above claims, wherein the support is made of regenerated cellulose derivatised with quaternary groups.

12. Use according to claim 11, wherein the quaternary groups are Q-groups.

13. Use according to one or more of the above claims, wherein the sample is applied in preparative amounts.

14. Use according to one or more of the above claims, for 2D electrophoresis.

15. Kit comprising a positively charged sample application support according to any of the above claims and an IPG gel.

16. Kit according to claim 15, wherein the IPG gel is a RTG-gel.

17. Kit according to claim 16, wherein the RTG-gel is an acidic interval RTG-gel.

18. Kit according to claim 17, wherein the acidic interval is pH 3.5-5.

19. Kit according to one or more of the claims 15-18, wherein the support is made of regenerated cellulose derivatised with Q-groups.

20. Sample applicator for electrophoresis, comprising regenerated cellulose derivatised with Q-groups.

ABSTRACT

The present invention relates to a novel use of a positively charged support, namely as a sample loading support for loading samples onto an electrophoretic gel. The support or paper is provided with positively charged groups, such as cation groups, and is used to load samples from the cathode side of the electrophoretic gel, preferably an IPG (Immobilised pH gradient) electrophoretic gel.

Furthermore, the invention relates to a kit comprising a positively charged sample application support as above and an IPG gel, preferably a pre-swollen RTG gel comprising an acidic pH-interval.